NASA-CR-197026

5P

Final Technical Report

Prepared: October 31, 1994

Title: NASA Ames Grant NAG 2 - 590
Muscle Injury and Repair in
Zero Gravity - Cosmos '89

Investigator Name: William T. Stauber, Ph.D.
Professor of Physiology
and Neurology

Institution: Department of Physiology
West Virginia University
Health Sciences Center North
Morgantown, WV 26506

Description:

A. Justification:

Skeletal muscle atrophy seen in actual or simulated weightlessness appears to be due to a combination of local and systemic factors - muscle activity, tension, and exogenous growth factors. Material properties of skeletal muscles may likewisebe weakened by exposure to zero gravity so as to predispose spaceworkers to mechanical strains and overload injuries. Little is known about muscle matrix changes following immobilization or weightlessness. From the studies on athletes who have been injured, immobilized, retrained and returned to sport with normal strength, there appears to be a increased risk of reinjury. Thus, the factors and conditions that lead to muscle atrophy and weakness may be different from those which regulate matrix organization and strength. In addition, if gravitational forces are necessary for connective tissue growth and development, then altered repair of muscle and connective tissues following injury might lead to muscle fibrosis and movement dysfunction. If this were to occur in injured spaceworkers, then normal movements might require additional energy utilization and emergency egress could be compromised.

B. Accomplishments:

Cosmos 2044 and Hindlimb Unloading

From our initial studies during Cosmos 2044, it appeared that hindlimb unloading induced a more rapid healing response than did spaceflight or ground based environments. However, a paradoxical situation seemed to exist: injured muscles may have healed more rapidly when subjected to hindlimb unloading but normal muscles from hindlimb unloaded animals were more fragile and ruptured more readily (e.g., upon landing or reambulation). To study these muscles, we have constructed a sophisticated computer-controlled rodent dynamometer and have tested the material properties of four tail-suspended rat hindlimbs. It appears from this limited sample that the rats have a reduced capability to withstand repeated strains as compared to control rats even though their muscles appear, on morphological examination, to have a relative proliferation of matrix material

Card done
AMS done

NOV 0 3 1994
To CASI

(Appendix 1). It is possible that the matrix around the atrophied fibers is structurally or chemically inferior to that of normal tissues. To investigate the cause of this tissue weakness, we collected a quantitative morphometric database of myofiber and matrix components from about 1,000 control, fibrotic, hypertrophic soleus and control and 2G gastrocnemius muscle fibers. This will serve as a normative base to compare samples from tail-suspended rats. From this experience, a routine methodological approach using computer image enhancement has been established for fiber areas and matrix content. The gastrocnemius muscles from the 2G NASA experiments contained smaller muscle fibers with increased extracellular matrix.

The remarkable changes seen in connective tissue physiology of skeletal muscle support similar findings for tendon and ligaments. However, the proliferation and degranulation of mast cells that occurs in the loose connective tissue in conjunction with matrix proliferation appear unique and may link mast cell degranulation with fibroblast's secretion of matrix materials. Interestingly, the mast cell response is common to both atrophy and hypertrophy of skeletal muscle.

In Cosmos 2044, the mast cell response was most exaggerated in animals subjected to 0 G. The numbers and apparent size of mast cells increased after injury. When mast cell degranulation was evident, the granules containing chymase often were free in the loose connective tissue and along the edge of myofibers which contained abundant amounts of fibronectin. The mast cell response continued for up to 12 days following injury.

Tail-suspended animals and dynamometry.

To explore the muscle tissue modifications due to hindlimb unloading and injury, we began physiological measurements using our custom-built dynamometer. The dynamometer was designed as a multiparameter testing device (isotonic, isometric and isokinetic) similar to that used to test humans (e.g., Kin-Com). It also allows the animals to be tested in a 30 degree head down position so that swelling of the muscles will not occur during testing. Preliminary results from dynamometry of four hindlimb unloaded rats by the method of tail suspension have been analyzed. Two conclusions can be made from the in vivo testing: 1) the muscles of the plantar flexor group were weaker following two weeks of suspension as indicated by a 50% reduction in work capacity; 2) the loss of force following three bouts of ten repetitive concentric and eccentric muscle actions (lifting and lowering) was greater than the loss of force due to the similar procedure in control animals indicating that the muscles from tail suspended animals were be more susceptible to material fatigue or strain injury. Although the number of animals tested so far was small, the results indicate that loss of strength following hindlimb unloading is accompanied by a decrease in material strength. Preliminary morphological evidence supports the hypothesis that muscles from hindlimb suspended animals are more susceptible to strain-induced damage.

These preliminary findings, if proven, have importance to human spaceflight and exercise prescription to prevent muscle strain injuries in man working in space.

Some additional observations are worth mentioning: 1) Tail suspended animals are more susceptible to death from anesthesia if they are tilted head upward for 5 minutes or more. If this applies to humans, then emergency surgery upon return to earth may have additional risks due to cardiovascular reflex abnormalities. 2) Muscles from tail-suspended rats swell when placed on a level surface. This means that measurements of muscle function must be performed in a head down position. If the animals are placed on a level surface for 30-60 minutes, the muscle swell and there is a measurable amount of wet weight change. Most of the swelling is located in the ECM but fibers also become round in shape. These observations cast doubt on the measures of extracellular fluid in muscle fibers from tail-suspended rats because head down position was not adequately controlled.

The remarkable changes seen in connective tissue physiology of skeletal muscle support similar findings for tendon and ligaments but it is still unclear why proliferation is common to both atrophy and hypertrophy. A theoretical model was developed to assess the effect of changes in myofiber cross-sectional area on the relative content of the extracellular matrix. The results reveal that the slow degradation rates of the collagens can lead to an apparent increase in matrix while the muscle actually atrophies. Further study is in progress to extend these observations and assess the functional significance.

One unusual finding, observed by one of my colleagues, was the evidence of myofiber pathology in one tail-suspended soleus muscle which was not injured. This sample demonstrated both hypertrophy and atrophy of fibers. Thus, a complicated mixture of muscle tissue responses may develop to tail-suspension.

Publications:

Stauber, W.T. 1989. A unique problem of muscle adaptation from weightlessness - the deceleration deficiency, In: Workshop on Exercise Prescription for Long-duration Space Flight. NASA Conference Pub. 3051, Harris, B.A. And Stewart, D.F. (eds), pp. 57-59. OK

Stauber, W.T., Fritz, V.K., Ilyina-Kakueva, T.E. and A.S. Kaplansky. 1991. Cosmos-2044 Rodent tissue repair (Experiment K-7-29): Skeletal Muscle. Cosmos 2044 Technical Memorandum, Mains Associates, Berkeley, CA, pp.xx-xx.

Stauber, W.T., Fritz, V.K., Burkovskaya, T.E. and E.I. Ilyina-Kakueva. 1992. Effect of spaceflight on the extracellular matrix of skeletal muscle after a crush injury. J. Appl. Physiol. 73 Suppl: 74S-81S.

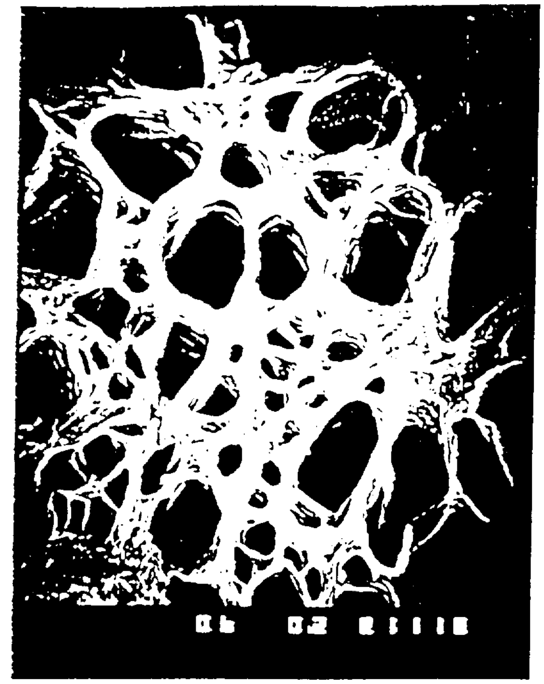
Stauber, W.T., Fritz, V.K., Burkovskaya, T.E. and E.I. Ilynia-Kakueva. 1993. Effect of injury on mast cells of rat gastrocnemius muscle with respect to gravitational exposure. Exp. Mole. Pathol. 59: 87-94.

Miller, G.R. and W.T. Stauber. 1994. Use of computer-assisted analysis for myofiber size measurements of rat soleus muscles from photographed images. J. Histochem. Cytochem. 42: 377-382.

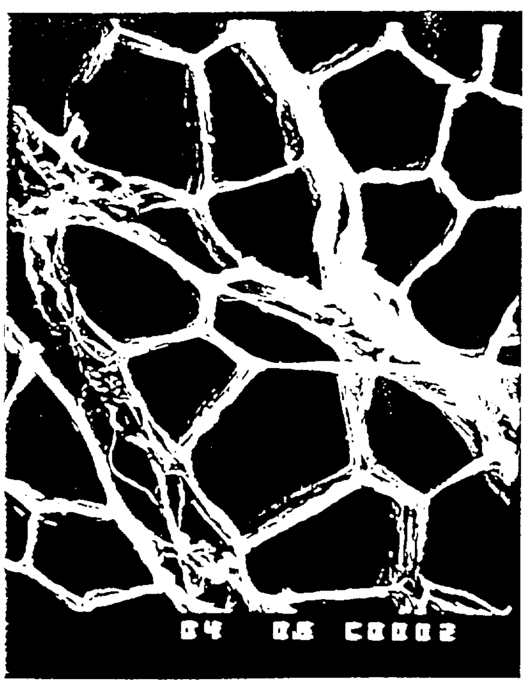
Stauber, W.T., Miller, G.R., Grimmett J.G., and K.K. Knack. 1994. Adaptation of rat soleus muscles to intermittent chronic strain. J. Appl. Physiol. 77(1): 58-62.

Stauber, W.T., Miller, G.R., and J.G. Grimmett. Adaptation of rat gastrocnemius muscles to 2 wk of centrifigation: Myofibers and Matrix. Aviation, Space Environ. Med. (Submitted).

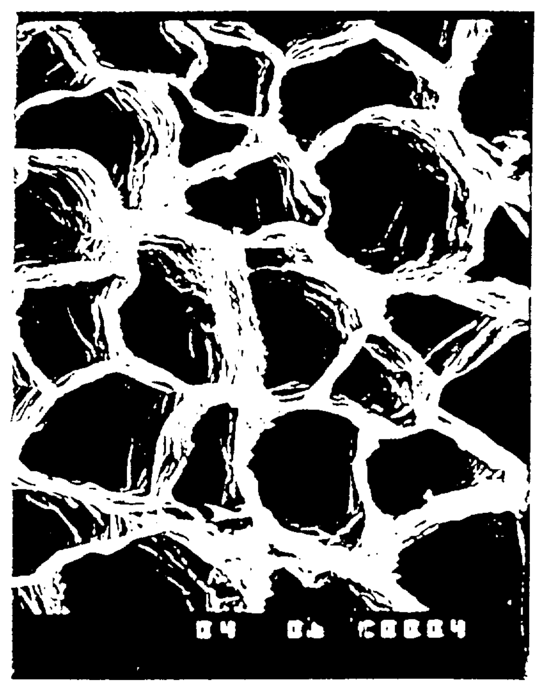
Control Soleus



Fibrotic Soleus



Control Soleus



Tail-Supsended Soleus

Appendix 1. SEM of connective tissue from soleus muscles.